

## Electron microscopy of gonococci in the urethral secretions of patients with gonorrhoea treated with penicillin and erythromycin

N. M. OVČINNIKOV, V. V. DELEKTORSKIJ, AND B. A. AFANAS'EV

*From the Central Institute for Research on Skin and Venereal Diseases of the Ministry of Health of the USSR*

The electron microscopic study of changes in gonococci in pus from patients before and during antibiotic treatment is of undoubted interest. Electron microscopy of ultrathin sections of urethral secretions shows up changes in the gonococcus itself and reveals details of the interrelationship between cellular elements in the pus and gonococci, both before and during treatment. It also allows study of the difference in the reaction of the cellular elements to antibiotic-sensitive gonococci and gonococci with reduced sensitivity to antibiotics.

With this in mind we examined pus from patients with gonorrhoea before and after they had been given an antibiotic.

### Material and methods

The urethral secretions from nine patients were used for study.

In four of them the gonococci isolated were sensitive to penicillin:

(1) *Male patient S* was suffering from acute gonorrhoeal urethritis and prostatitis. He had been ill for 18 days, after an incubation period of 7 days, and it was his first attack of gonorrhoea. The organism was sensitive to 0.05 unit penicillin.

(2) *Male patient RRG* was suffering his first attack of acute gonorrhoeal urethritis. The organism was sensitive to 0.1 unit penicillin.

(3) *Male patient PA* was suffering from acute gonorrhoeal urethritis and epididymitis. He had been ill for 17 days after an incubation period of 12 days and it was the first time he had had gonorrhoea. The organism was sensitive to 0.1 unit penicillin.

(4) *Male patient M* had acute gonorrhoeal urethritis.

In five patients the gonococci showed reduced sensitivity:

(5) *Male patient Lu.N*, who had had gonorrhoea a year before, had been ill for 18 days after an incubation period of 2 days. His acute urethritis had failed to respond to four injections of 600,000 units bicillin III\* intramuscularly. The gonococci were sensitive to 0.4 unit penicillin.

(6) *Male patient Ku* had acute gonorrhoeal urethritis. He had been ill for one month after an incubation period of 5 days. Before admission to hospital he had been given 600,000 units bicillin III daily for 4 days, 10 g. chloramphenicol, 10 g. tetracycline, 300,000 units crystalline penicillin intramuscularly every 3 hours to a total dose of 4.2 m.u., and an intramuscular injection of a pyrogen, all without effect. The gonococci were sensitive to 0.6 unit penicillin.

(7) *Male patient Ka* had acute gonorrhoeal urethritis which relapsed after treatment with erythromycin\*. The gonococci were sensitive to 0.6 unit penicillin.

(8) *Female patient T* had acute urethritis and endocervicitis. The gonococci were sensitive to 0.5 unit penicillin.

(9) *Male patient VG* was suffering his first attack of acute gonorrhoeal urethritis. He had been ill for 2 weeks after an incubation period of 7 days. The gonococci were sensitive to 0.9 unit penicillin, but in spite of this reduced sensitivity, the patient responded rapidly to penicillin.

Before treatment characteristic gonococci were seen inside and outside the cells in all these patients in Gram-stained urethral smears. Samples were taken before treatment and 30 minutes and 3 to 6 hours after the drug had been given. In parallel, Gram-stained smears were examined under the light microscope. When gonococci were not found under the light microscope (this happened mostly 3 to 6 hours after the drug had been administered), the material was not examined under the electron microscope.

The material was processed for electron microscopy as previously described (Ovčinnikov and Delektorskij, 1971).

\*400,000 units erythromycin = 0.476 g.

The work was partly supported by the WHO

Received for publication August 31, 1973

\*Bicillin III contains benzyl penicillin, procaine penicillin, and benzathine penicillin in equal proportions

## Results

### BEFORE TREATMENT

Electron microscopy of ultrathin sections of the urethral secretions of patients with penicillin-sensitive gonococci revealed both intracellular and extracellular gonococci. Around the extracellular gonococci many globular and elongated structures were visible. The outer wall was three-layered and the cytoplasmic membrane was normal. There were many ribosomes. The beginning of absorption of gonococci by epithelial cells was well marked and the pseudopodia were all turned towards the gonococci. There were no round and elongated structures in the part of the gonococci adjacent to the epithelial cell (Fig. 1). Inside the epithelial cells the gonococci were situated in the cytoplasm. In places phagosome membranes were visible (Mph). The gonococci were unchanged and contained a large number of ribosomes. Some gonococci inside the phagosomes of the polynuclears were undergoing lysis (Fig. 2). In the majority it was only the spherical structures round them that were lysed (Fig. 3). In Fig. 2 the contents of the granules in a polynuclear may be seen escaping into the phagosome.

In one patient (PA: No. 3) a Gomori test for acid phosphatase was carried out. The deposition of lead round some phagocytosed gonococci was noted and that metal was also encountered in some places in the cytoplasm. There was great activity of the proteolytic enzymes in the area of a phagocytosed gonococcus.

Roughly the same picture was observed in electron microscopy of gonococci with reduced sensitivity to penicillin. The difference lay in the fact that many of the gonococci were somewhat deformed. This increase in polymorphism is characteristic of forms with reduced sensitivity. Most of them were otherwise unchanged and the globular structures round the gonococci were well preserved. The absorption of the gonococci by epithelial cells was somewhat more marked (Fig. 4).

In the patient VG (No. 9), who was infected with a less sensitive strain but nevertheless responded to treatment with penicillin, the pattern shown by electron microscopy before treatment was also similar to that in the cases with sensitive gonococci, but individual diplococci situated inside phagosomes lost their ribosome structure; they became larger with vague outlines.

In cultures of gonococci from these patients, even after only 24 hours (Fig. 5), large specimens (G') were found in which the whole cell had a nuclear structure and there were many deformed gonococci with altered mesosomes (Fig. 6, Ms).

### AFTER TREATMENT

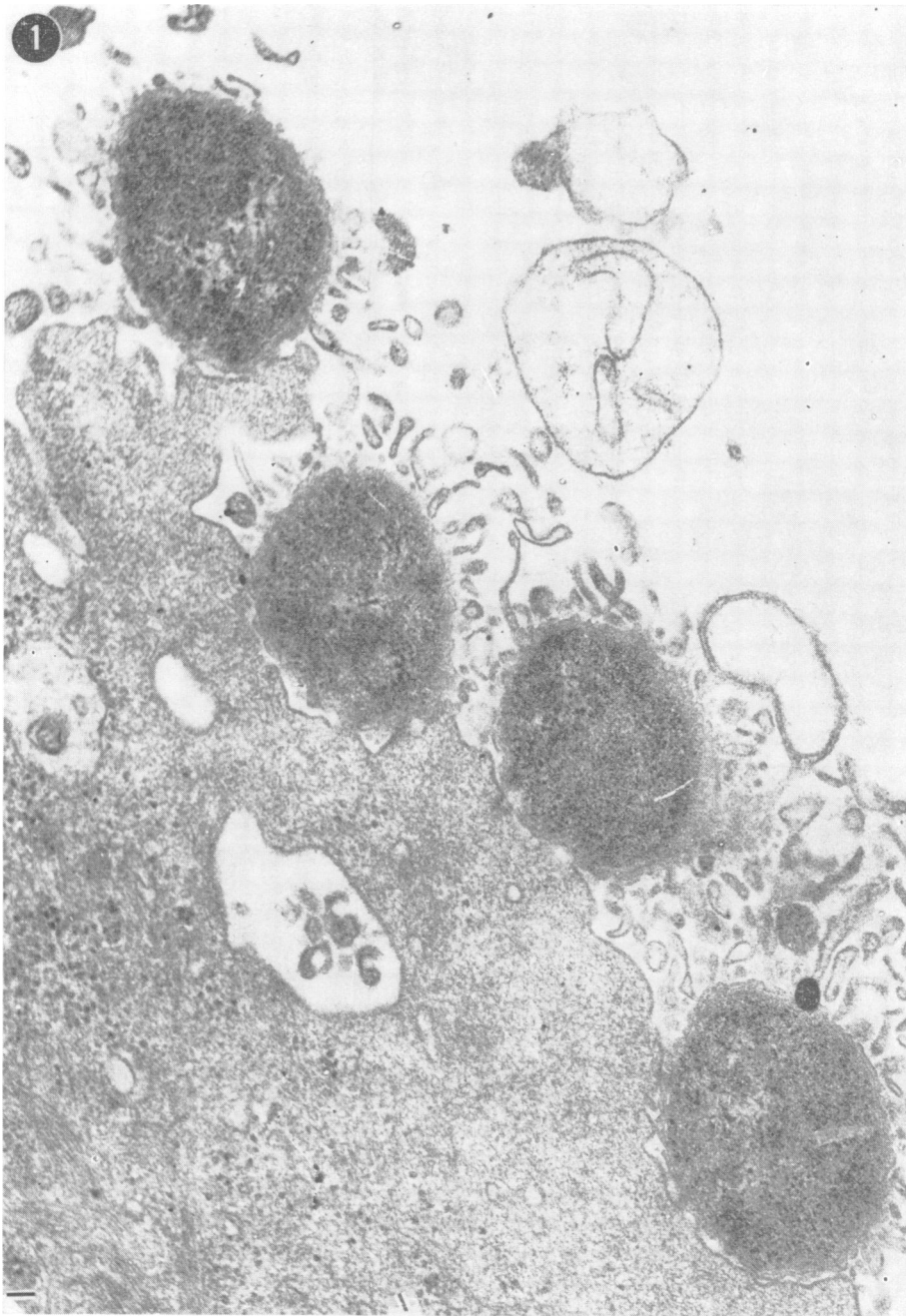
30 minutes after the administration of 800,000 units or 400,000 units of erythromycin (Patients S (No. 1) (Fig. 7) and M (No. 4)) or a single intramuscular injection of 200,000 units of crystalline penicillin (Patients RRG (No. 2) and PA (No. 3)), there were more leucocytes with phagocytosed gonococci. In some of the gonococci situated inside the phagosomes of the polynuclears the globular structures were affected (Fig. 7, detail) and the outer wall had become less well defined. Some of the diplococci were unchanged but occasional ones were in various stages of lysis. Inside the epithelial cells the gonococci were unchanged. No essential difference was found between the appearance of gonococci from patients treated with penicillin and those treated with erythromycin.

3 hours after the administration of antibiotics, large phagosomes were present in some polynuclears. They have a clearly marked wall and contain remnants of gonococci (Fig. 8). Apparently it is the cytoplasmic membrane of the gonococci which persists for the longest time. In some phagosomes the gonococci were preserved more or less unchanged (Fig. 9). In one patient (PA: No. 3) gonococci were visible under the electron microscope after 3 hours, although only occasional diplococci were encountered in Gram-stained smears.

After 6 hours electron microscopy showed many phagosomes with a large number of residual bodies (apparently lysed gonococci) in the polynuclears.

After 30 minutes, in patients with reduced sensitivity of gonococci to penicillin (Lu.N (No. 5), Ku (No. 6), Ka (No. 7), and T (No. 8)), there were considerably more leucocytes with phagocytosed gonococci. In the extracellular gonococci the drop-like structures became bigger and took on a granular appearance. Pseudopodia were clearly visible in the polynuclears. In some phagocytosed gonococci the stratification of the outer wall was clearly visible and no changes at all could be seen in the structure of the gonococci. Some of the diplococci were lysed, only the membrane and a residual body being preserved. The gonococci on and in the epithelial cells were distorted (Fig. 11).

After a dose of 400,000 units erythromycin had been taken by Patient Ku (No. 6), at first no changes could be seen in the extracellular gonococci. When the material obtained after 3 hours was examined the impression was gained that it contained more globular and elongated structures round the gonococci. The gonococci themselves were deformed and there were mesosomes which were also somewhat deformed. In this same patient, 6 hours after administration of erythromycin, the stratification of the outer



**FIG. 1** *Ultrathin section of urethral secretion from patient RRG (2) before treatment. Gonococcus on an epithelial cell at the beginning of absorption. In the parts of the gonococcus adjoining the epithelial cell, there*

*are no globular or elongated structures. The cell membrane under the gonococcus is unchanged. There are abundant spherical structures around the gonococcus.  $\times 20,000$*

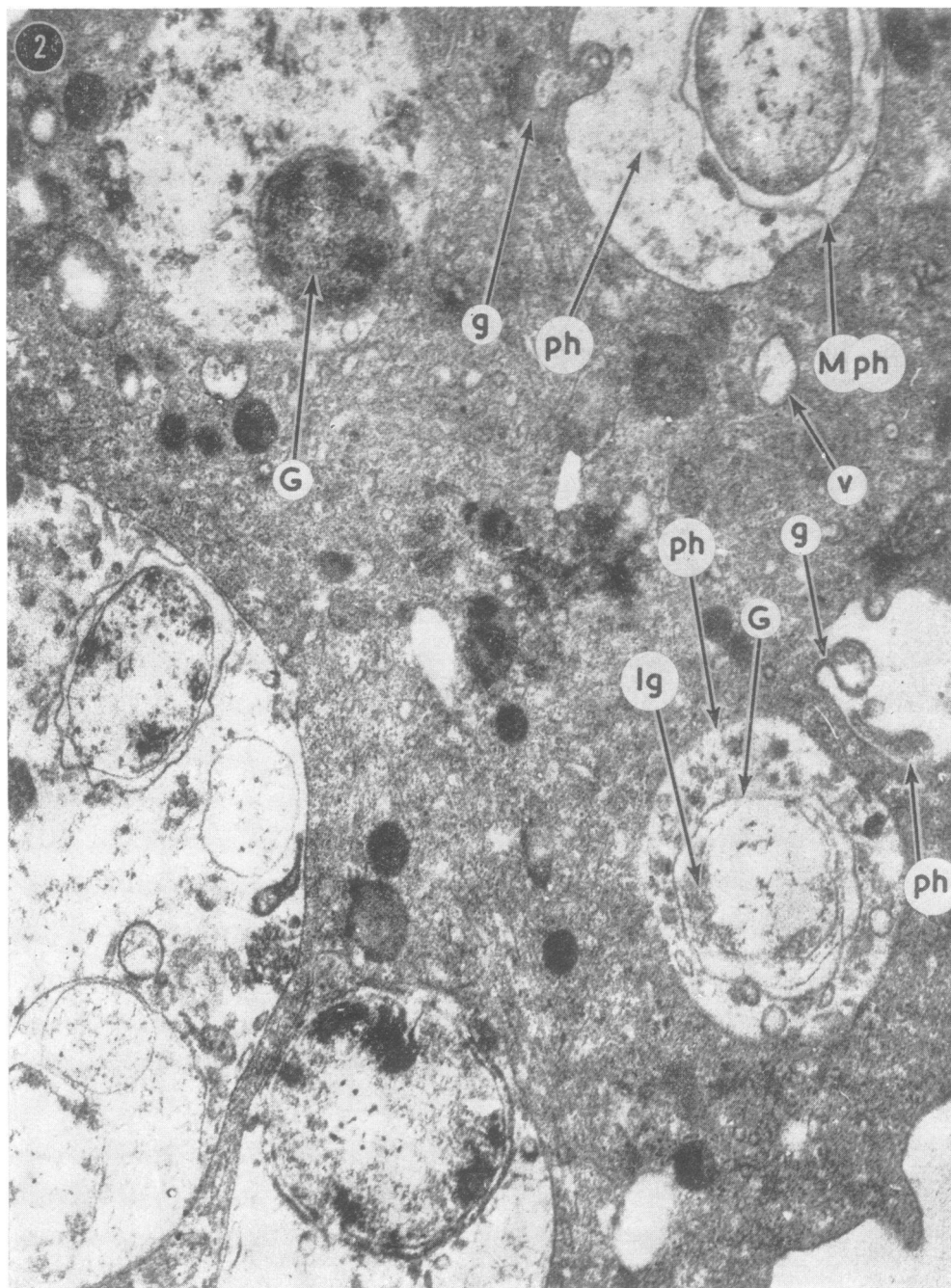


FIG. 2 Ultrathin section of urethral secretion from patient RRG (2) with acute gonorrhoea, before treatment. Large phagosomes with clearly marked membranes (Mph), some of which contain remnants of

lysed gonococci (lg). In the phagosome the contents of the granules of the polynuclear are escaping into the phagosome (ph). g—granules, v—vacuoles, G—gonococci.  $\times 25,000$



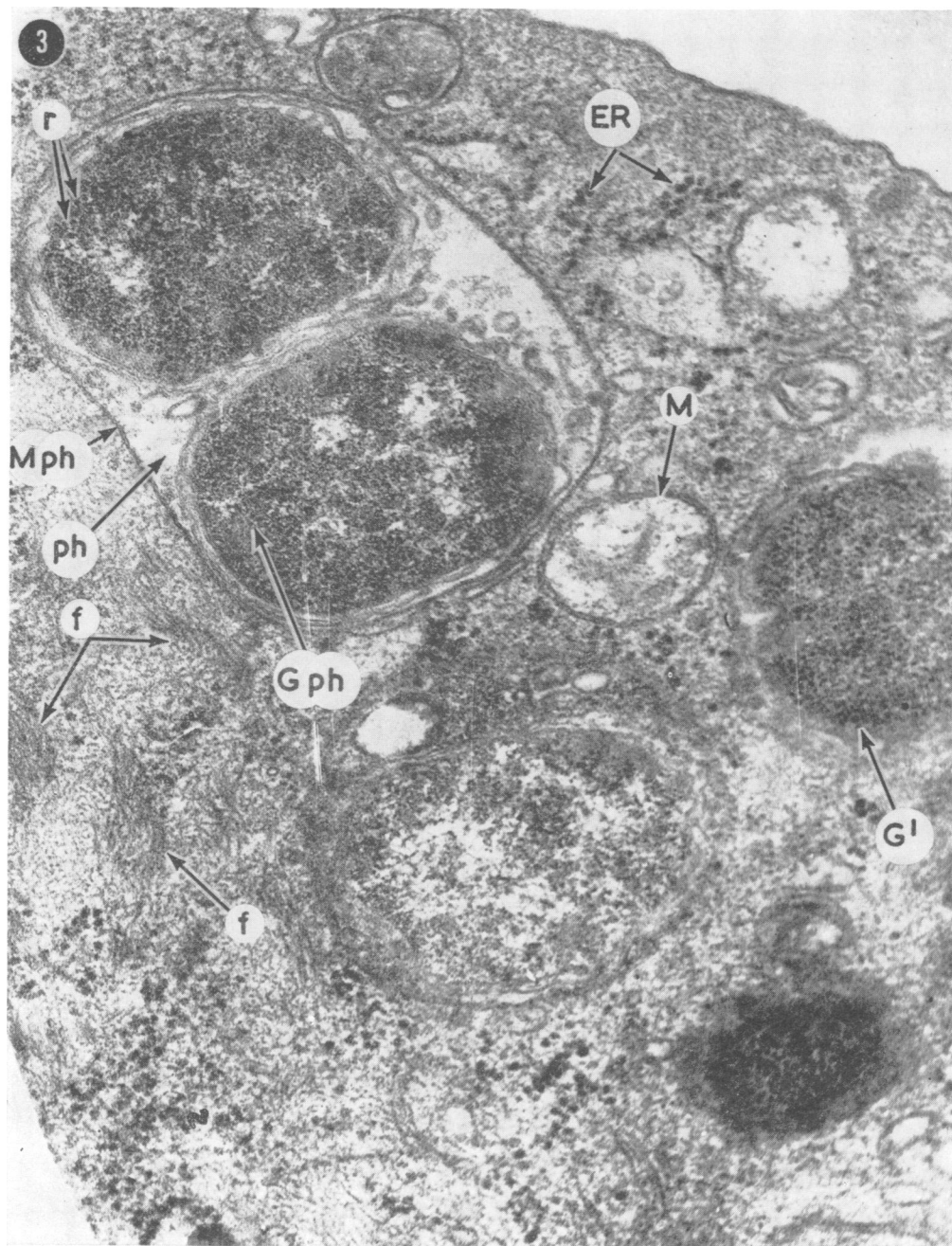


FIG. 3 Ultrathin section of urethral secretion of patient RRG (2) before treatment. Gonococci are seen both inside the phagosome (Gph) and in the cytoplasm proper (G'). They are unchanged.

M—mitochondrion with a double membrane. Fine filaments in groups (f), ribosomes (r), endoplasmic reticulum (ER). No particular change can be seen in the gonococcus in the cytoplasm itself.  $\times 40,000$

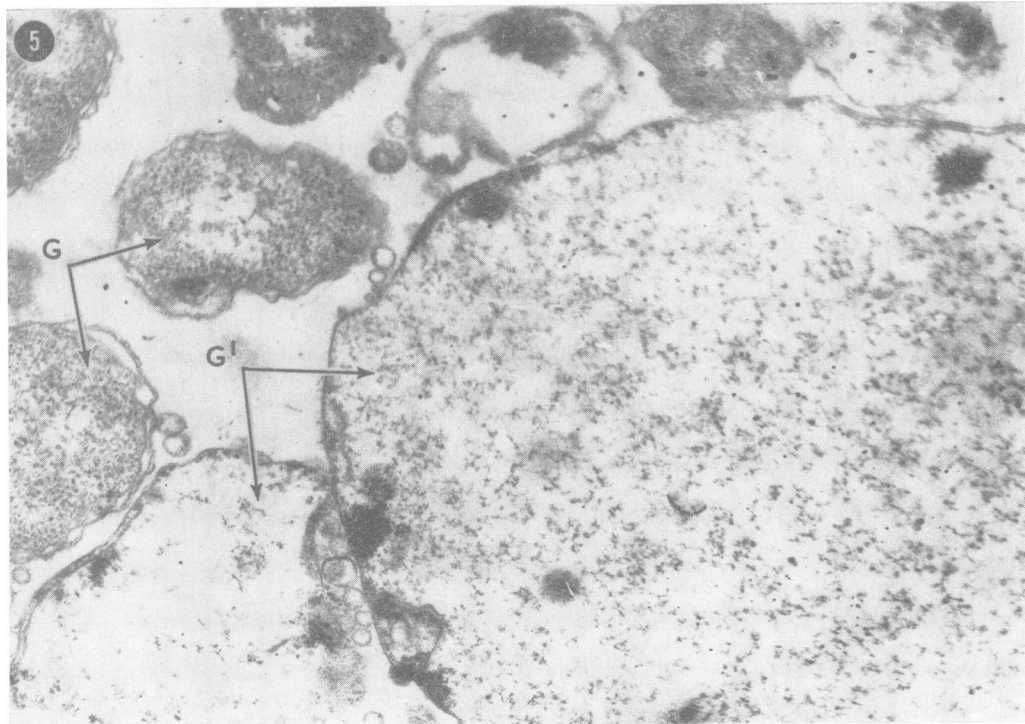
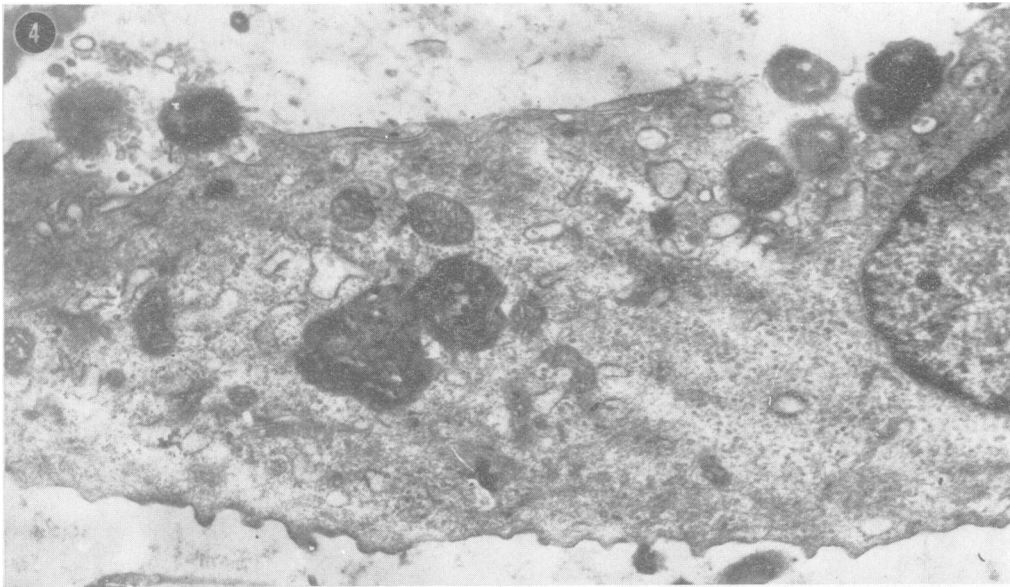


FIG. 4 Ultrathin section of urethral secretion from patient Ku (6) before treatment. Absorption of gonococci by an epithelial cell. The gonococci both inside and outside the cell are unchanged. Vacuoles approach the site of absorption closely.  $\times 8,000$

FIG. 5 Ultrathin section of day-old culture on ascitic agar, pH 7.4, using material from patient Ku (6). Tendency to formation of L-forms. Giant specimens (G') of gonococci side-by-side with small specimens (G)

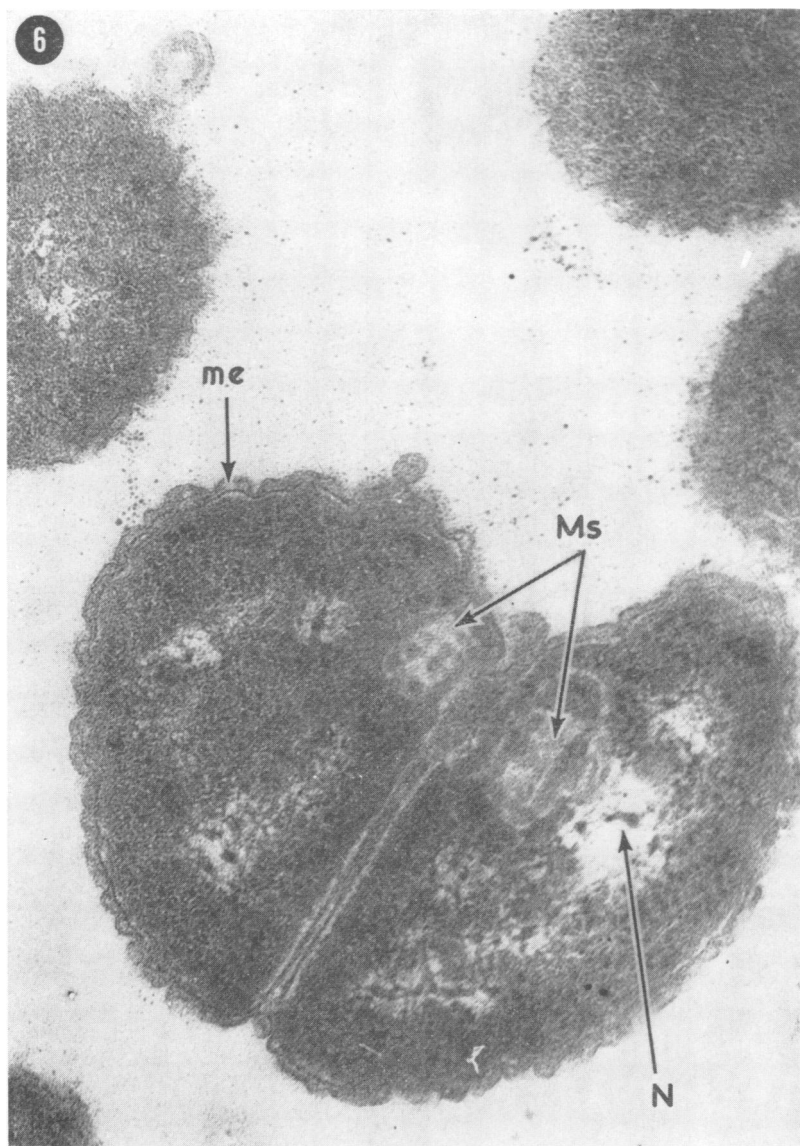
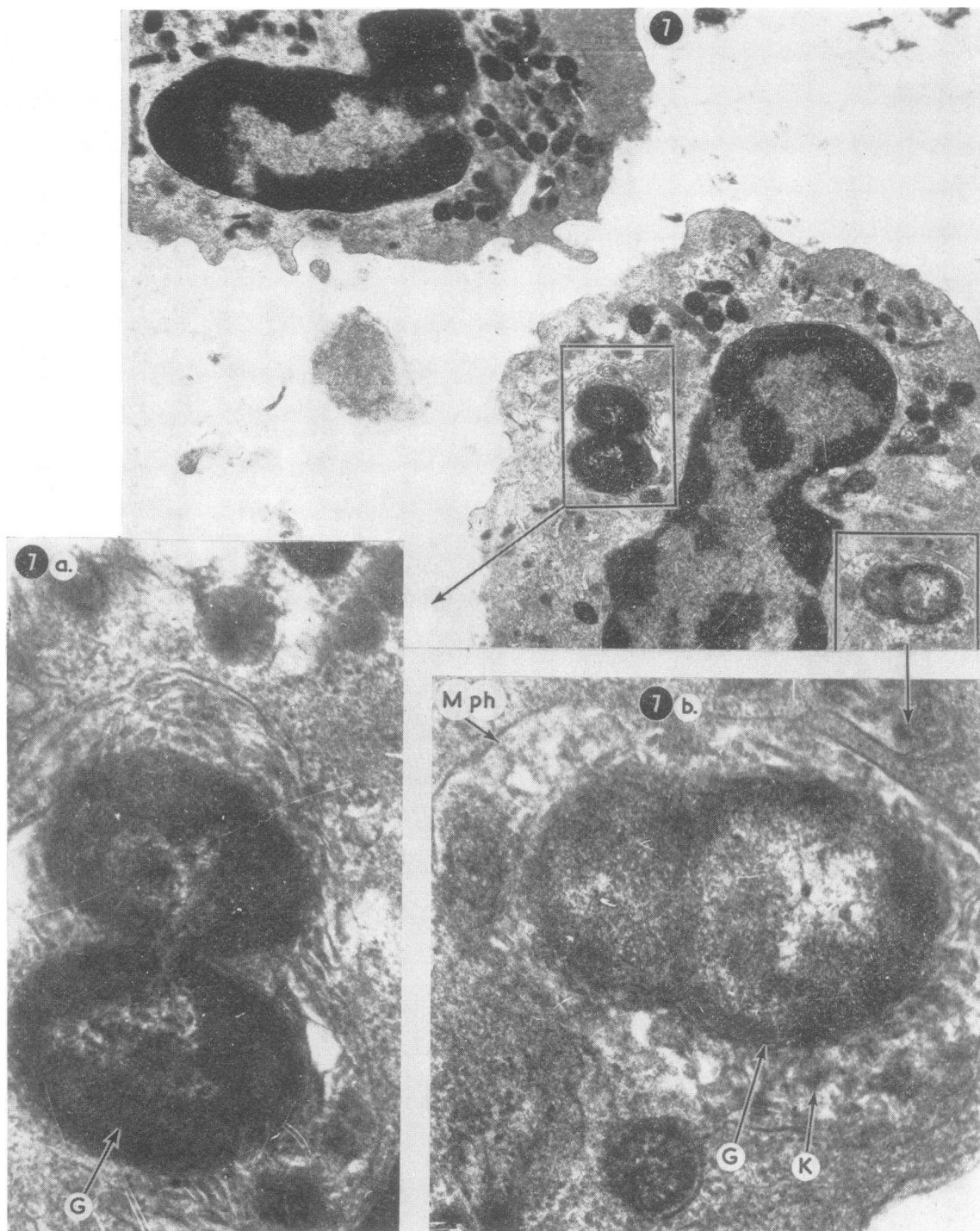


FIG. 6 Section from same culture as Fig. 5, showing deformation of the gonococcus. The mesosome (Ms) has undergone change. The nuclear substance

is distributed in various places in the cell with compact granules of DNA. Giant forms distorted. me—outer wall, N—Nucleus





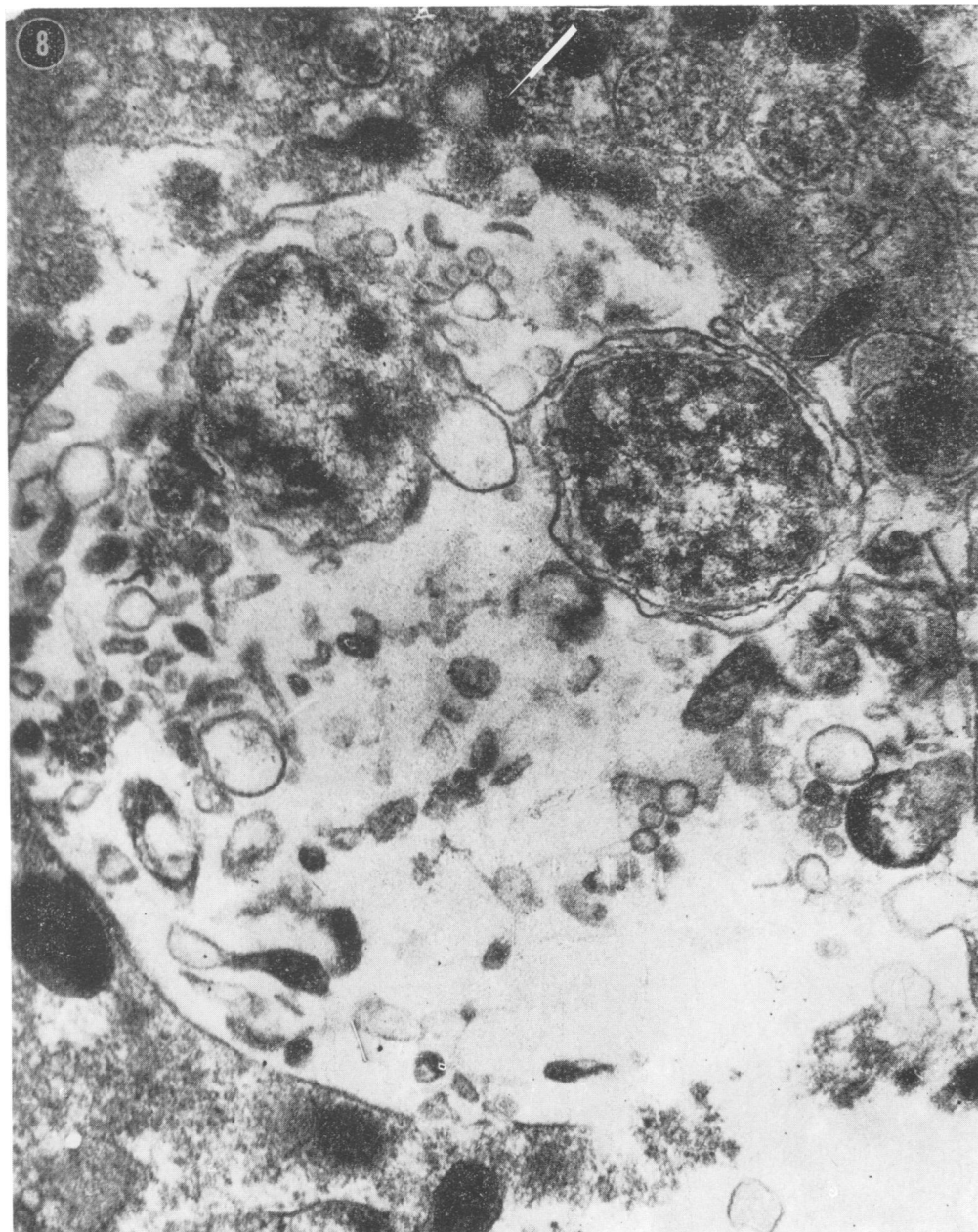


FIG. 8 Ultrathin section of urethral secretion from patient RRG (2) 3 hours after a single intramuscular injection of 200,000 units penicillin. A gonococcus inside the phagosome is undergoing lysis. The outer

wall persists and the cytoplasm in certain sectors has no clear boundaries but is distributed throughout the cell. Spherical structures are scattered throughout the phagosome. Phagosome membrane clearly outlined

FIG. 7 Ultrathin section of urethral secretion from patient S (1) 30 minutes after he had been given 800,000 units erythromycin.  $\times 8,000$ . (a) and (b)  $\times 40,000$  G—gonococcus, Mph—phagosome membrane, K—spherical structures around gonococcus

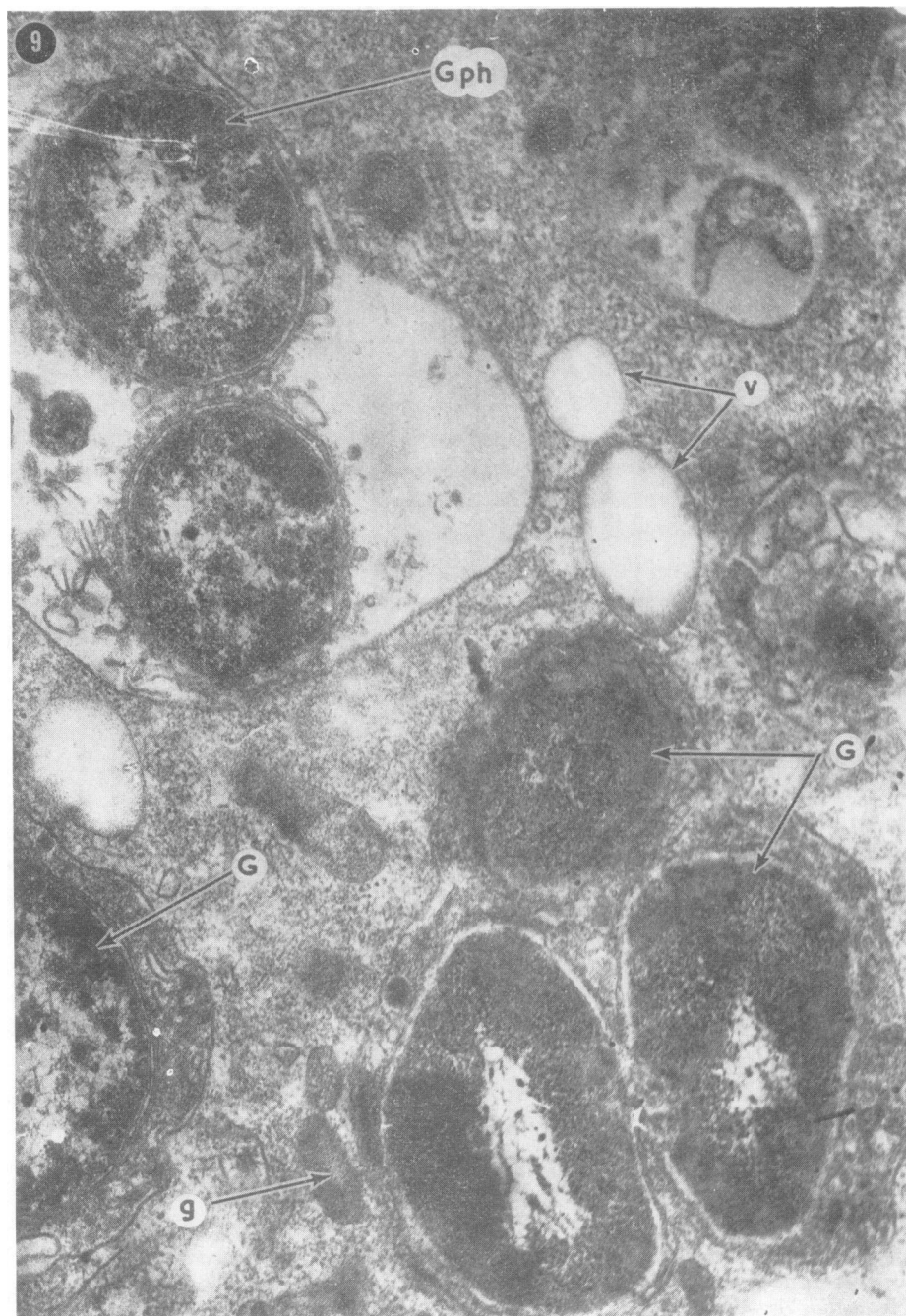


FIG. 9 Ultrathin section of urethral secretion from patient RRG (2) 3 hours after administration of 200,000 units penicillin. There are unchanged gonococci inside one phagosome (Gph), while in another

there are changes in the outer wall, and the spherical structures around the gonococcus (G) have lost their shape. v—vacuoles, g—granules.  $\times 35,000$



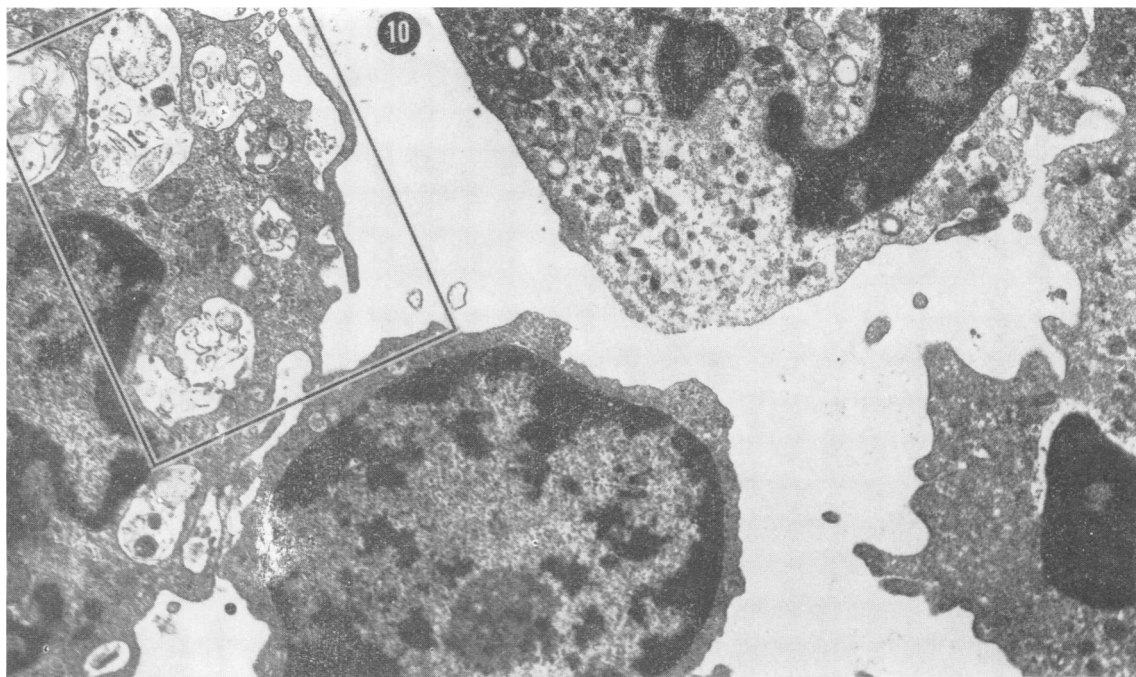


FIG. 10 Ultrathin section of urethral secretion of patient RRG (2) 6 hours after administration of penicillin. The cells contain many lysosomes and vacuoles with numerous types of residual bodies. The

impression is given that the gonococci have been lysed and that only the residual bodies remain (see detail in 10a overleaf)

wall was seen in greater relief and the small globular structures round the gonococci had begun to become granular. In gonococci phagocytosed by polynuclears the outer wall was thin and most of them contained no globular structures and only occasional mesosomes. Some gonococci were lysed. The nuclear vacuole was of the ordinary type with DNA filaments. In the epithelial cells the gonococci were unchanged but distorted.

In Patient Lu.N (No. 5), 3 hours after a single administration of 200,000 units crystalline penicillin, the gonococci inside the phagosomes in the polynuclears had kept all their structural features and division of the gonococci was clearly visible. The gonococci in the cytoplasm itself also maintained their structure. The round structures had disappeared but a homogenous mass remained around the gonococci (Fig. 12). There were many pseudopodia. The impression was gained that lysis had begun in some diplococci. On and inside the epithelial cells the gonococci were deformed but no special changes were visible.

The same pattern was observable in Patients Ka (No. 7) and T (No. 8).

At 6 or 9 hours after treatment no gonococci could be found under the electron microscope in the discharge of most of the patients. The polynuclears still had a large number of big phagosomes, some of which contained residual bodies. In some patients (e.g. Ku (No. 6) (Fig. 13)), 6 hours after treatment, unchanged gonococci at the division stage were to be found in polynuclears which had numerous phagosomes inside them. The extracellular gonococci also maintained their characteristic structure.

A comparison of ultrathin sections of the urethral secretions taken from patients before and during treatment with penicillin or erythromycin and containing gonococci sensitive to penicillin or with reduced sensitivity to that antibiotic, showed that there were no substantial differences between the two types before treatment when the gonococci showed little change.

During the treatment of sensitive strains with penicillin, a large number of lysed gonococci are

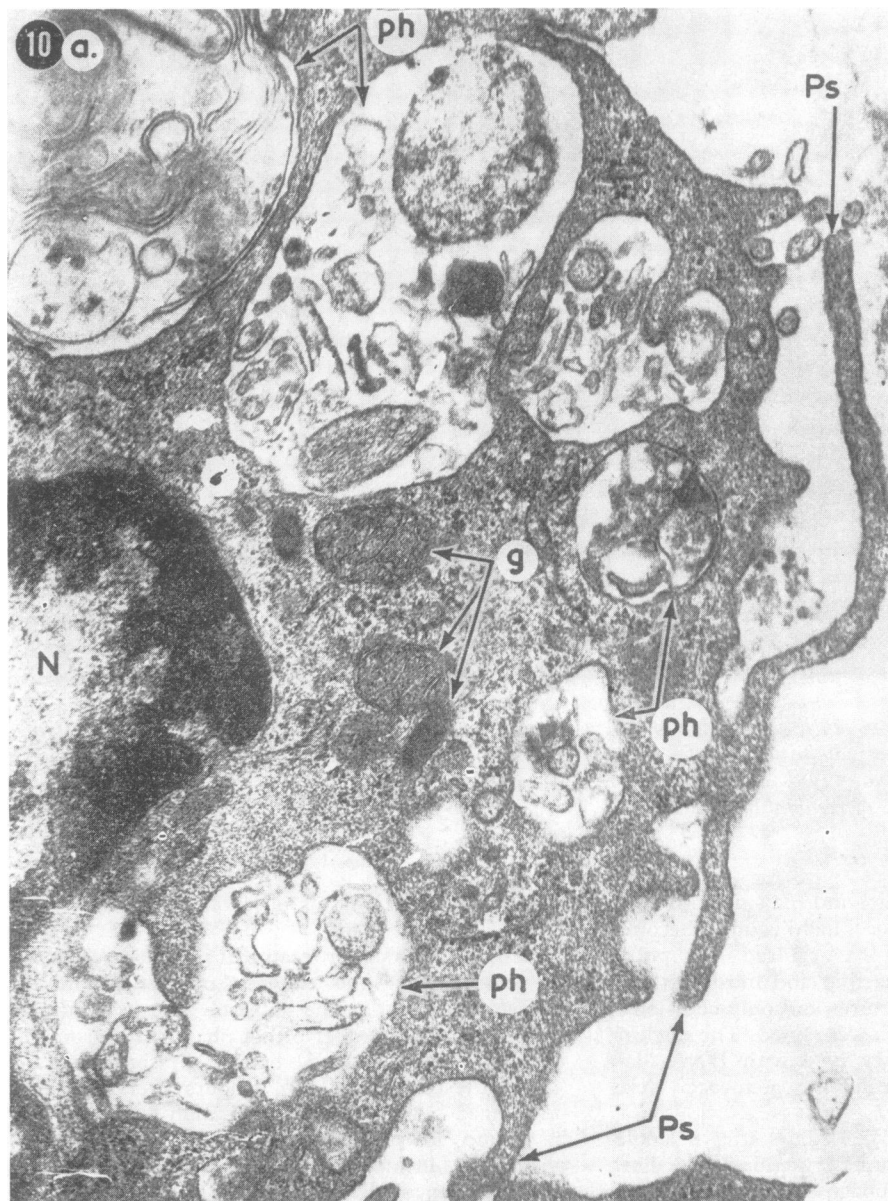


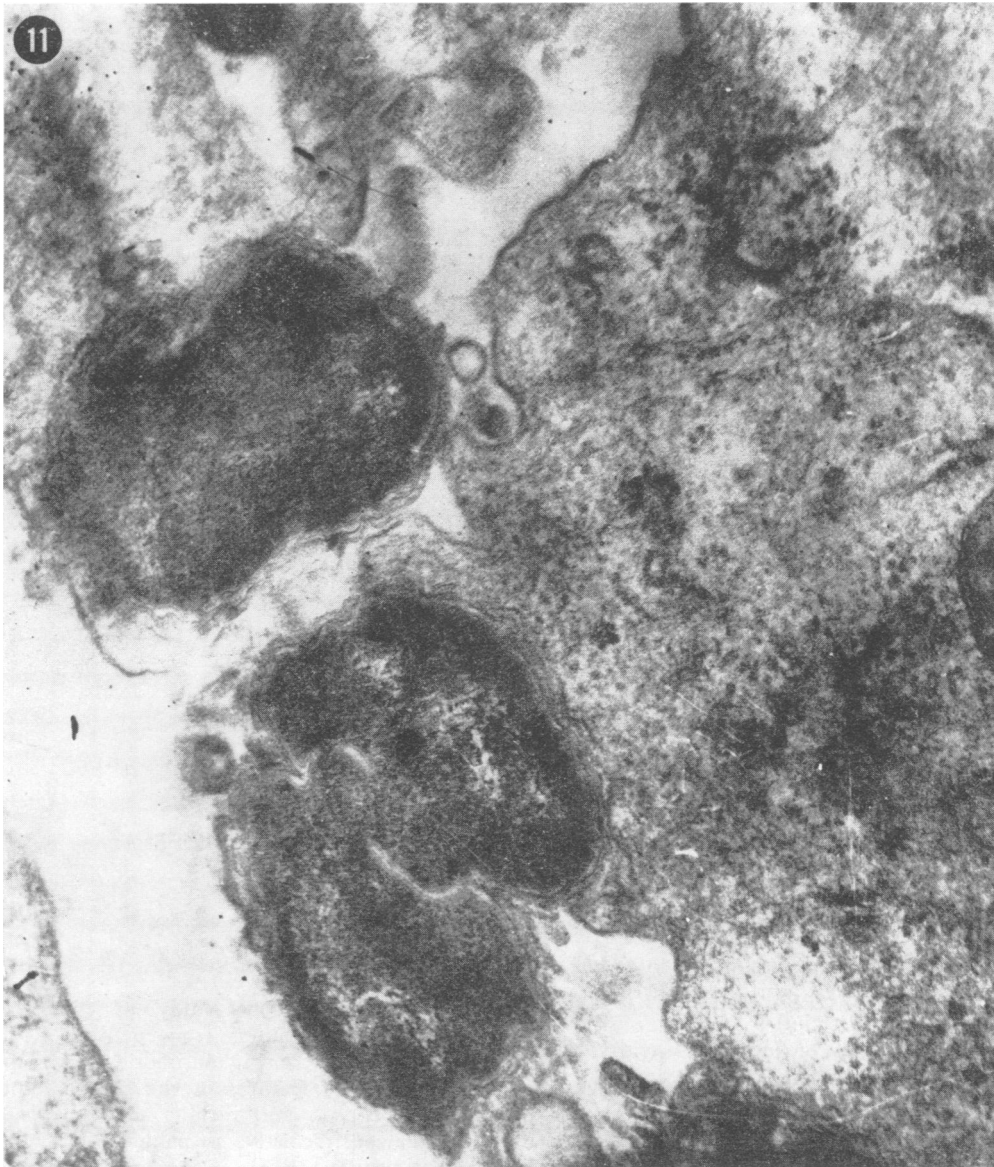
FIG. 10a Detail of Fig. 10.

*N*—nucleus

*Ps*—pseudopodia

*ph*—phagosome

*g*—granules



**FIG. 11** *Ultrathin section of urethral secretion from patient Lu.N (5) 30 minutes after administration of 400,000 units erythromycin. Fewer and less clear-cut*

*spherical structures are seen round the gonococcus. The gonococci are deformed but have maintained their structure.  $\times 45,000$*



FIG. 12 *Ultrathin section of urethral secretion of patient Lu.N (5) 3 hours after a single intramuscular injection of 200,000 units penicillin. The*

*polynuclear has numerous pseudopodia and there are elongated granules.  $\times 10,000$*

observed in the polynuclears. They disappear earlier. Transfers on to nutrient media produce growth of gonococci of normal size and shape. Later (after 6 hours) a considerable number of leucocytes with phagosomes filled with residual bodies can be seen.

Material from patients in whom the gonococci are less sensitive contains a large number of globular structures. During treatment many monstrous, deformed gonococci are observed. Increased absorption of gonococci by the epithelial cells is noted, whereas apart from deformation no other changes are observed in gonococci in the epithelial cells. Preparations from cultures examined under the electron microscope also contained a considerable number of monstrous forms and partly changed mesosomes. Among gonococci phagocytosed by polynuclears there were fewer lysed specimens and in some cells the gonococci were unchanged and continued to divide.

### Conclusions

- (1) An electron microscope study of ultrathin sections of urethral secretions from patients with gonorrhoea before and during antibiotic treatment revealed a number of features of the body's fight against the gonococcus, and various morphological changes in gonococci sensitive to penicillin or with reduced sensitivity.
- (2) The sensitive strains were characterized during treatment by marked lysis of gonococci inside the polynuclears. Gonococci with reduced sensitivity to penicillin showed marked polymorphism and a tendency to L-transformation which was maintained in cultures. In pus incomplete phagocytosis predominated and the gonococci showed no particular change.
- (3) In the process of treatment with penicillin and erythromycin, activation of the phagocytic activity



**FIG. 13** *Ultrathin section of urethral secretion of patient Ku (6) 6 hours after administration of erythromycin. A polynuclear with large phagosomes, one containing a gonococcus at the division stage,*

*without visible changes, and residual bodies. The extracellular gonococcus is also unchanged and has numerous spherical structures around it.*

of the polynuclears was observed. This was shown by the appearance of a large number of pseudopodia, by the increase in the number of leucocytes with phagocytosed gonococci, by the inclusion of granules in the phagosomes, and by the increase in intracellular lysis.

(4) In the epithelial cells gonococci maintained a clear-cut structure and lysis was not observed.

(5) During successful treatment with penicillin or erythromycin, the unchanged gonococci disappear and a large number of big phagosomes with residual bodies in the polynuclears appear.

### Summary

Ultrathin sections were prepared from the urethral secretions of patients with gonorrhoea both before and during penicillin and erythromycin treatment to study the morphology of the gonococcus and its inter-relationship with cellular elements under the electron microscope.

It was established that gonococci in pus have some morphological features that differ from those of gonococci obtained from cultures. Gonococci inside leucocytes and epithelial cells did not undergo any particular changes and no differences were noted between gonococci sensitive to penicillin and those with reduced sensitivity.

Under the influence of penicillin incomplete phagocytosis becomes complete. Gonococci with reduced sensitivity remain for a longer period in the pus during treatment and take on monstrous shapes. The structure of the mesosomes changes but that of the gonococci does not undergo any particular alteration. 3 to 6 hours after the administration of penicillin the antibiotic-sensitive gonococci had

disappeared from the secretions and only residual bodies were found in the phagosomes.

Penicillin and erythromycin treatment are associated with increased phagocytosis.

### Reference

- OVČINNIKOV, N. M., and DELEKTORSKIY, V. V. (1971)  
*Brit. J. vener. Dis.*, **47**, 419

### Étude au microscope électronique des gonocoques des sécrétions urétrales chez des gonococciques traités par la pénicilline et l'érythromycine

#### SOMMAIRE

Des coupes ultra-fines furent préparées à partir de sécrétions urétrales de malades gonococciques, aussi bien avant que pendant le traitement à la pénicilline et à l'érythromycine, ceci pour étudier au microscope électronique la morphologie du gonocoque et les relations existantes avec les éléments cellulaires.

Il a été constaté que les gonocoques du pus avaient quelques caractéristiques qui différaient de celles des gonocoques de culture. Les gonocoques à l'intérieur des leucocytes ou des cellules épithéliales n'ont pas subi de modification particulière et il ne fut noté aucune différence entre les gonocoques sensibles à la pénicilline et ceux de sensibilité réduite.

Sous l'influence de la pénicilline, la phagocytose incomplète devient complète. Pendant le traitement, les gonocoques de sensibilité réduite restent plus longtemps dans le pus et prennent des formes monstrueuses. La structure des mésosomes change mais celle des gonocoques ne subit aucune modification particulière. Trois à six heures après l'administration de la pénicilline, les gonocoques sensibles à l'antibiotique avaient disparu des sécrétions et on ne trouva que des éléments résiduels dans les phagosomes.

La phagocytose augmente pendant le traitement à la pénicilline et l'érythromycine.